Flow Cytometry Core
NIAMS, NIH

Protocol for staining for apoptosis using Annexin V (FITC or APC) and Propidium Iodide or 7-AAD

Uses and Rationale:
One way a cell can die is to undergo apoptosis, also commonly referred to as programmed cell death. When this occurs many changes take place inside and outside of the cell. Flow cytometry can detect apoptosis because when a cell becomes apoptotic, it expresses a phosphatidyl serine residue on the extracellular cell surface. Annexin V recognizes and binds to this residue, and with the inclusion of a viability marker, one can differentiate cells that are live, dead and apoptotic.

Materials:
Annexin V (FITC or APC)
Viability dye – either PI or 7AAD
Calcium containing PBS or Annexin V binding buffer

Method:
1. Spin cells down, wash 1x w/ calcium containing-PBS (PBS MUST have calcium – if you use calcium free PBS, you may have just ruined your experiment!)

1a. If you are staining cells for surface markers, stain them here…..30 minutes on ice in dark for all surface markers. Wash 1x with Ca-containing PBS or 1x binding buffer, and proceed.

2. In 500uL of the binding buffer, at room temperature (not in cold/on ice!!) add Annexin V dye (5uL of Caltag, either FITC or APC, works well).

3. Wait 10 minutes, add PI (5uL of a 50ug/mL stock solution in PBS) or 7-AAD (stock solution is 1mg/mL, add 2 uL of dye for every 100uL PBS volume).

4. After 10 minutes after adding viability dye, analyze your cells

Important technical notes:
Note: If you wait longer than 60 minutes from adding Annexin V to analyzing, you will not be able to analyze your cells. This is a VERY time sensitive expt. which must be done at room temperature, in dark, and always always always with calcium containing medium

Both BD and Caltag have protocols on their websites that are useful references as well.